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HEREDITY AND VARIATION IN THE SIMPLEST ORGANISMS [1]

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UNICELLULAR animals present all the problems of heredity and variation in miniature. The struggle for existence in a fauna of untold thousands showing as much variety of form and function as any higher group, works itself out, with ultimate survival of the fittest, in a few days under our eyes, in a finger bowl. For studying heredity and variation we get a generation a day, and we may keep unlimited numbers of pedigreed stock in a watch glass that can be placed under the microscope.

Work in this field, so far as it has yet been carried, gives in simple form results which are typical of the trend of investigation over the entire subject; it gives a sort of diagram of the main facts of heredity and variation. For this reason it appears worth while to present here the main results in their bearing on general questions. Technical accounts of the investigations have been published elsewhere,[2] but these are rather forbidding, owing

[1] A paper read before the Scientific Association of Johns Hopkins University.

[2] Jennings, H. S., "Heredity, Variation and Evolution in Protozoa." I, "The Fate of New Structural Characters in Paramecium, with Special Reference to the Question of the Inheritance of Acquired Characters in Protozoa," *Journ. Exper. Zool.*, 5, 1908, 577–632. II, "Heredity of Size and Form in Paramecium, with Studies of Growth, Environmental Action and Selection," *Proc. Amer. Philosophical Soc.*, 47, 1909, 393–546.

to the mass of statistical data involved. For the evidence of the statements here made the reader is referred to these.

Unicellular organisms are essentially free germ cells —germ cells that are subjected to the immediate action of the environment, both direct and selective. For long periods they propagate without that intercrossing which so tremendously complicates the study of heredity in higher animals. Here if anywhere we should see readily the effects of environment and of selection in modifying a race.

Let us look first at the direct action of the environment: the "inheritance of acquired characters." It has commonly been thought that under the conditions found in these organisms "acquired characters" are readily inherited. This is because the progeny arise by division of the parents; they are therefore the *same* as the parent. It would seem a matter of course therefore that they should have the same characteristics as the parent, however these characteristics arose.

But when we examine just what occurs in the production of the new individuals, we find—as usually happens when we look closely at biological processes—that the thing is not so simple after all. We find that in reproduction the characteristic features of the parent disappear[3] and are produced anew in the offspring. Thus in Paramecium (Fig. 1) the characteristic form of the ends, the oral groove, the shape of the body—these disappear during fission, and reappear in the growth of the young. In Stylonychia (Fig. 2) all the appendages are absorbed at division; they appear anew in the young, in their characteristic structure, number and distribution, by a process comparable to the development of organs in a higher animal.

[3] Certain exceptions to this, of no theoretical importance, are mentioned in the original papers. Certain characters sometimes pass directly to *one* of the offspring, but their multiplication is of course always by new production.

Reproduction in these creatures may then be compared to the dissolving of a crystal in its mother fluid; on recrystallization the new crystal appears with the same form and angles as the parent. But it is really a new

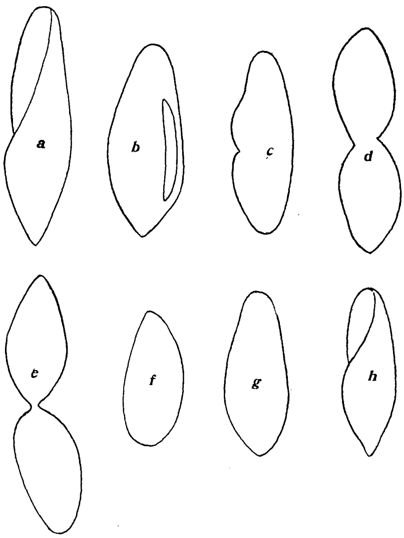

FIG. 1. Changes in form shown by Paramecium during reproduction. *a*, form of adult; *b* to *e*, successive stages of fission; *f*, *g*, immature young; *h*, young after reaching adult form.

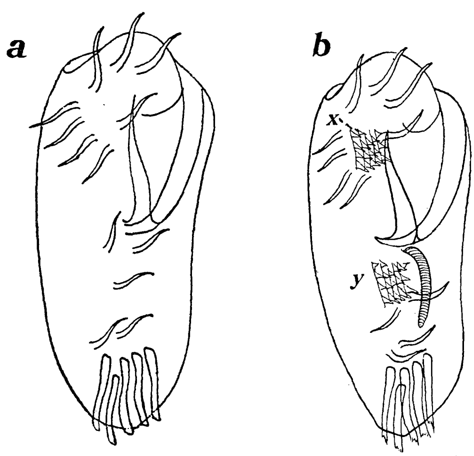

FIG. 2. Stylonychia, after Wallengren. *a*, adult, showing the appendages; *b*, stage preparatory to fission. At *x* and *y* have appeared the beginnings of the new sets of appendages of the two progeny to result from fission, after the disappearance of the old appendages.

crystal, with new-formed angles. The analogy of reproduction with recrystallization is striking in many ways, as we shall see farther.

Thus inheritance is, here as elsewhere, not transmission but new production. The question of heredity then is: What characters will thus be produced anew? Will the progeny reproduce *any* character that the parent happens to have?

When we study the matter in such an organism as Paramecium (Fig. 1), we find that all the characters common to the race—the "normal" characters—are regularly reproduced. But how about characters that are not typical; characters that have been produced in the individual parent by the environment; "abnormal" characters, and the like? It is easy to produce such new characters by environmental action, and it is easy to find in certain cultures individuals that present unusual features. Specimens with altered form, with new appendages, with differently arranged parts, are not very rare. Many of those found in natural cultures correspond in appearance to what we might expect of a *mutation.*

Will such untypical characters reappear in the progeny, so that we shall get a race with the new characteristic?

Examination of a large number of cases in Paramecium shows that these untypical characters are never reproduced in the young. Sometimes such a thing as an appendage may pass bodily to one of the progeny, just as a parasite clinging to the outer surface might do; but there is no multiplication of such a character; no tendency to produce a race bearing it. The young reappear in the form typical for the race, without regard to the individual peculiarities of the parent.[4]

What is produced in the new generation therefore depends on the fundamental constitution of the race, not on the accidental form of the parent. Again the analogy

[4] For many examples of this, with figures, see the first of the papers already referred to.

with crystallization forces itself on us. The characteristic form of crystals is easily changed; by filing off the angles we might convert a large number of pyramidal crystals into the quite new form of cubes. But if we dissolve these and allow them to recrystallize, we obtain, not cubes, like the parents, but the original crystalline form characteristic for that particular chemical compound.

If we should modify the chemical constitution of the substance, it would then crystallize in new shapes. If we could modify the fundamental constitution of the organism we should probably find it likewise appearing in new forms. Whether this occurs at times in unicellular organisms we shall ask later. But it is important to grasp the fact that it does not occur often nor easily; that the ordinary activities of life do not observably bring it about; that the mere presence of a new character in the parent has no evident tendency to produce such a result. Many of the untypical forms found in Paramecium were such as one might imagine due to an alteration in the fundamental constitution of the race (a mutation?), but the new characteristic was not reproduced in the progeny.

But besides the untypical or "abnormal" characters of certain individuals, there are the common differences among individuals that are fully "normal." In the Protozoa, as in all organisms, differences in size and proportion among differing individuals are common; *variation* is the rule here as everywhere. We must then examine these differences, under the question already set forth: What characters are produced anew in reproduction? Will the progeny produce anew these diversities of the parents, in such a way that from large parents arise large progeny, from small parents small progeny, from intermediate parents intermediate progeny?

In Paramecium we find individuals differing greatly in size. From a "wild" lot of Paramecia we isolate such differing individuals and propagate from them, all under

the same conditions. We find that many of these differences *are* inherited; from large individuals we get large races; from small individuals small ones. We find, then, that Paramecium consists of many races, differing from each other in mean size slightly but constantly. Eight of these different races were isolated and propagated for hundreds of generations; some were carried through several complete "life cycles." Each such race consisted of specimens all derived from a single parent individual. Unquestionably many other races exist, that could be isolated by proper means.

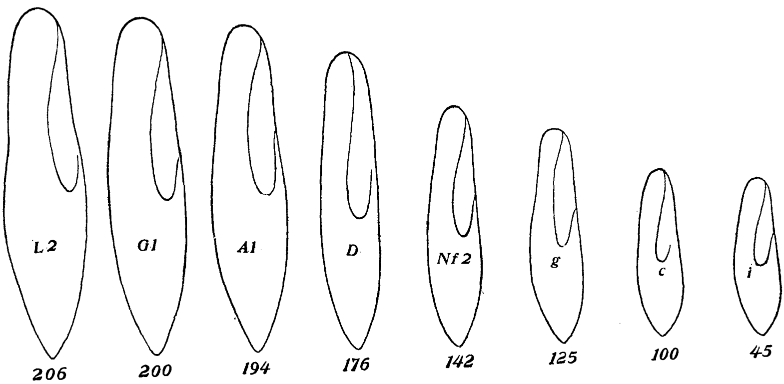

FIG. 3. Diagram showing the relative mean lengths of the eight different races of Paramecium that were isolated. The magnification is about 340 diameters. The actual mean length of each race is given in microns below the corresponding outline.

Fig. 3 is a diagram showing the relative mean lengths of the eight races isolated, as determined by measuring at intervals lots of 100 or more individuals of each race. The mean length for any race is constant under given conditions. The differences between adjacent races are very slight; thus, between the races *c* and *i* of the diagram the difference in mean length was but five to seven microns or .00028 inch. For measuring such constant differences between races even the "fourth decimal place of the biometrician," so heavily contemned of late, would seem to be required. This gives us something of a measure of the minuteness of the steps by which evolu-

tion may occur, if we hold that one of these races has arisen from another.

We find then that by selection we can isolate many races of different mean size, and that the relative mean size is inherited in each race.

But another fact of equal importance comes forth. Within each race (derived from a single parent) the size of the different component individuals varies extremely. The largest specimens of a given race are more than twice as long as the smallest specimens, and every intermediate dimension occurs. We may therefore represent the composition of a single race by the diagram of Fig. 4. These

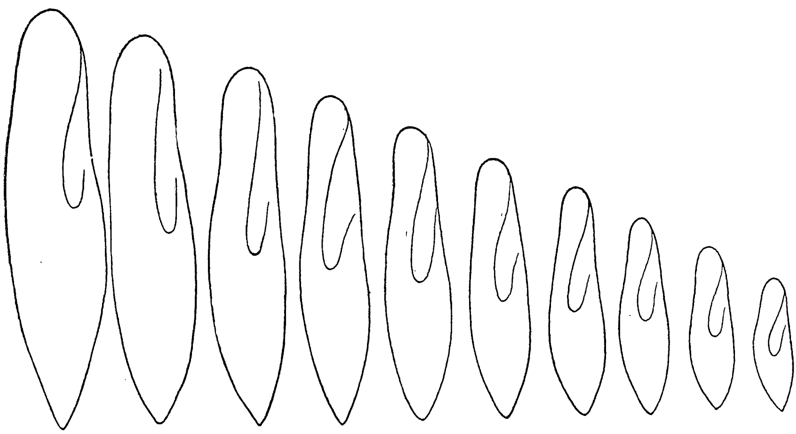

FIG. 4. Diagram of a single race, showing the variations in the size of the individuals. The race represented is *D* of Fig. 3, and the magnification is the same as in Fig. 3 (340 diameters). The individuals vary from 80 to 256 microns in length.

differences in size are due to growth, to amount of nutrition, and to other environmental conditions; a detailed analysis of the action of these factors is given in the second of the two papers above referred to.

Now we come to the most important point. Are the varying sizes within the single race inherited? Will large specimens produce large progeny, small ones small progeny, so that from a single race we can get several, of differing sizes? And can we by repeated selections of

the largest individuals for breeding steadily increase the mean size of a race?

Breeding from the extreme specimens—the largest and smallest—of a single race, we get several hundred individuals from each. *Both produce progeny of the same mean size.* Each produces a whole series of varying individuals, just like the original racial series (Fig. 4); the series produced by the largest individual is exactly like that produced by the smallest, or by any other. The differences between the individuals within such a series are due to growth and environment. Such differences are not inherited: *the race breeds true,* without regard to the peculiarities of the individual parent. A great number of such breeding experiments were carried out, in which selection was continued for many generations, but the results were invariably the same. *Selection within the pure race is of no effect on the size.*

Furthermore, marked differences in the parents due to different environments become quickly equalized in the progeny when the environments are made the same. Thus environmental effects are not inherited. Neither selection nor environmental action changes the size of the pure race.

Thus in our study of the "normal" variations we come to the same result as in our previous study of abnormalities, new characters, apparent mutations, and the like. What is produced in inheritance depends, not on the evident external features of the parent cell, but on the fundamental constitution of the race. Each race has its own peculiar constitution, and under different conditions this same constitution gives rise to various sizes and forms, producing thus the variations within a race, illustrated in Fig. 4. But all these different individuals of a race are potentially the same; at the same age and under the same conditions throughout, all would be alike.

Now consider again the species as a whole: in this

case Paramecium (*aurelia* or *caudatum* or both).[5] We have found it made up in the way indicated in Fig. 5. It consists of a series of many races, differing in mean size; while each race is made up of a series of individuals,

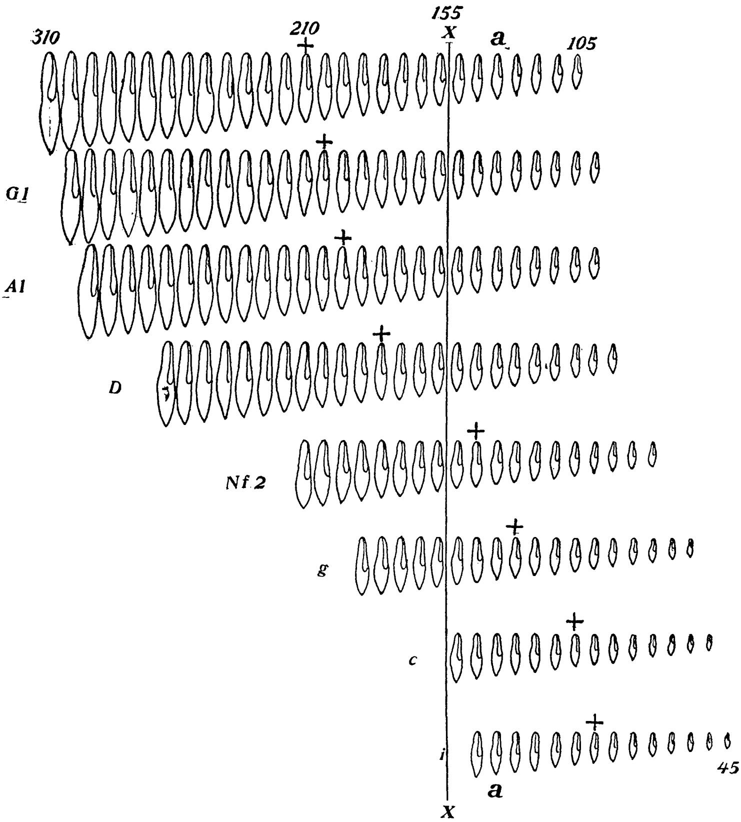

FIG. 5. Diagram of the species of Paramecium, as made up of the eight different races of Fig. 3. Each horizontal row represents a single race. The individual showing the mean size in each race is indicated by a cross placed above it. The mean of the entire lot is shown at *x*-*x*. The numbers show the measurements in microns. The magnification is about 43 diameters.

that are of varying size, though potentially alike. Reference to this diagram will help to understand certain fundamental facts of variation, heredity and the effects of selection in these organisms.

[5] For a discussion of the species question in Paramecium see the second of the original papers, pp. 498–500.

As the diagram shows, individuals of the same size are found in many different races. An individual of the size shown at *a* might belong to any one of the eight races. Thus from individuals of the same size we may get many different results in breeding. Similarly, individuals of very different size (as the extremes of any horizontal series) may produce progeny of identically the same character. We can not tell by its external characters to what race a specimen belongs; breeding is the only test. In higher organisms, as is well known, we often find a similar state of affairs.

How will selection act on such a complex species? As we have seen, selection within a single race is without effect. But if we make selections among the individuals of a mixed collection of races, such as Fig. 5 shows, we reach most instructive results. By making our selections in the proper way, we for a time make steady progress toward a certain goal. We will suppose that we do not know of the existence of these races; this is the case with most experiments in selection. From the species as a whole, as shown in Fig. 5, we will select for increased size. Let us follow the old plan of selecting many individuals showing the desired character; we will preserve all specimens above the mean size of the entire collection. That is, we divide the collection at $x - x$ rejecting all those to the right. By so doing it is evident that we exclude all specimens of the two smallest races *c* and *i*, while preserving the majority of the specimens of the larger races. Allowing these to propagate, we of course get a mixture of the remaining larger races; hence the mean size of the whole collection will be greater than at first. Selecting again those above the mean size of this lot, we drop out another small race, and the mean of the collection as a whole again rises a little. We are making good progress in the improvement of our species. By taking successive steps of this character, dropping out the smaller races, first partly, then completely, one after another, we can for a long time continue to improve by

selection. But finally we reach a stage in which all but the largest race have been excluded. Thereafter we can make no farther progress. In vain we choose for breeding the largest specimens of the lot; all belong to the same race, so that all produce the same progeny. Selection has come to the end of its action.

It is well known that this is a course of events commonly observed in selective breeding. Improvement occurs for a time, then stops. We might have produced the final result at once, in our infusoria, by merely isolating at the first selection the largest individual of the entire lot; its progeny would have produced at once a pure race of the largest size attainable. Selection here consists simply in isolating already existing races; it produces nothing new.

Thus the facts in Paramecium furnish an excellent illustration, in the simplest possible form, of the principles of breeding for improvement so convincingly set forth in de Vries's recent work on plant breeding, and in his other writings.

It is well known that in inheritance extremely marked parental characters appear less marked in the progeny; the progeny of extreme parents are on the whole nearer the mean than were the parents. This fact is spoken of as regression. The reason for this is clear in such a collection as Fig. 5. Suppose we breed from the very largest specimens of the entire collection. These are the largest individuals of the largest race. They produce, as we have seen, like any other specimens of that race, progeny of merely the mean size for that race. The progeny will then of course be on the average smaller than their parents, but they will be above the mean size of the species as a whole, since they belong to the largest race. Thus "regression is not complete"; the progeny diverge from the parents toward the general mean of the species, but do not reach it. If however we consider a single pure race alone (Fig. 4), and breed from the ex-

tremes, then "regression is complete," since the progeny are of the mean size for the race as a whole.

Work with "pure lines"—where no intercrossing of races or individuals occurs—is possible with but few organisms, and little of it has been done. In the few investigations carried on in this way, the same conditions have been found that we have set forth above for Paramecium. They were first shown by Johannsen[6] to hold for beans and barley, and later by Elise Hanel for Hydra.[7] The fact that there exist diverse races, tending to breed true, has of course been shown for many species, but in most cases it is difficult to maintain pure lines, and thus to absolutely demonstrate the relations above set forth, as has been done for beans, barley, Hydra and Paramecium. In the Protozoa it has of course been possible to carry the work through an immensely greater number of generations than in many celled organisms. The extremely important work of Barber[8] has shown the same condition of affairs to exist in yeast and bacteria, though with certain additional factors to be mentioned later.

When we deal with organisms that continually intercross, the conditions of course become immensely complex. Each individual represents, as it were, many diverse lines, whose appearance and disappearance in the hide-and-seek way characteristic of Mendelian inheritance makes interpretation extremely difficult. There is not wanting evidence that the same principles are at work even here; that the results of the study of "pure lines" give the clue by the aid of which the more complex results of selection in biparental inheritance may be unraveled. If this is the case, then selection would act even here also by isolating diversities that already exist, not by producing diversities. There are of course some not

[6] Johannsen, W., "Erblichkeit in Populationen und in reinen Linien," 68 pp., Jena, 1903.

[7] Hanel, Elise, "Vererbung bei ungeschlechtlicher Fortpflanzung von *Hydra grisea*," *Jenaische Zeitschr.*, 43, 1907, pp. 321–372.

[8] Barber, M. A., "On Heredity in Certain Micro-organisms," *The Kansas University Science Bulletin*, 4, 1907, 1–48, pl. 1–4.

yet thoroughly analyzed facts that are difficult to interpret on this basis, so that its general adequacy remains to be determined.

The work with Protozoa emphasizes further certain important points regarding *variations*. The interest of studies in variation lies mainly in the assumption that the variations are heritable, supplying material for selection and evolution; this assumption is openly or tacitly made in most work on the subject. Yet when we actually determine how far this is true, we find (as we have seen) that in a pure race of infusoria *all* the differences between individuals are environmental and without significance in inheritance. If we study these variations by biometric methods and laboriously work out numerical coefficients of variation (as the author has done on a large scale in his original paper) we acquire data which are perhaps not without some sort of interest, but which have no bearing on inheritance or selection or evolution. The "standard deviation" and "coefficient of variation" express in a pure race mere temporary conditions, of no consequence in heredity. If we could make all conditions of growth and environment the same throughout our pure race, all the evidence indicates that the standard deviation and coefficient of variation would be zero, and this is the positive value of their assistance in determining what shall be the characteristics of the progeny.

Even when we deal with mixtures of diverse races the coefficient of variation is, as a rule, mainly determined by differences due to growth and environment. "Wild" cultures of Paramecium, consisting of many races, may not give higher coefficients of variation than those obtained from pure races. The coefficient of variation is then by no means an index to the permanent heritable differentiations in a collection; its value may be high when there are no such differentiations, or low when such differentiations exist. The important question for all such work is: What diversities are heritable, what are not? This is an experimental question, and the rôle of statis-

tics in answering it is a most subordinate one. When an author deals in standard deviations and coefficients of variation, the first question the reader should ask is: Has the author determined what part of the diversities thus measured have any bearing on heredity? They may have absolutely none.

Thus the mere fact that observable variations exist between individuals can not properly be appealed to as furnishing material for selection and evolution, as has been so generally done. Most such variations have in the organisms studied absolutely no bearing on the evolutionary process, and there seems little doubt but that this is true for organisms in general.

Illustrations of the practical bearing of these points may be found without departing from the particular organism with which we are dealing. It has been shown that the two products of the division of a single individual Paramecium often differ in size at a given time, so that "variation" occurs in non-sexual as well as in sexual reproduction.[9] But these "variations" are mere temporary fluctuations, without effect in heredity, so that their relation to evolution is *nil*. Again, Pearl[10] showed that conjugants are less variable than non-conjugants. This is true even within the limits of a single race, as I can confirm from extensive studies. But all the variations in such a case, both in the conjugants and non-conjugants, are purely temporary matters, without effect on posterity; so far as evolution or heredity or selection goes they can be left quite out of account.[11]

Comparative studies have often been made of the variability of higher animals under different methods of reproduction, under different conditions, etc.; the varia-

[9] Simpson, J. Y., "The Relation of Binary Fission to Variation," *Biometrika*, 1, 1902, 400–404. Pearson, K., "Note on Dr. Simpson's Memoir on *Paramecium caudatum*," *Biometrika*, 1, 1902, 404–407.

[10] Pearl, R., "A Biometrical Study of Conjugation in Paramecium," *Biometrika*, 5, 1907, 213–297.

[11] This of course is no criticism of Pearl's paper, which is one of the foundational ones for this line of work. When different races are present, the less variability of the conjugants is of the greatest significance.

bility of parthenogenetic generations has been compared with that of sexual generations, and the like. These have no meaning for evolutionary questions unless we know whether any of the diversities are heritable. In general, it would appear that most observed variations are *not* heritable.

Perhaps more important even than the distinction between temporary modifications and really heritable differences is another point regarding "variations." Even leaving aside the temporary modifications, much discussion of variation assumes that the word implies an actual *change* from one condition to another. This is obviously a very different matter from mere observance of two different conditions in different individuals. If our object is to discover how far we have actually observed evolution *taking place,* the distinction between variation as an active change and variation as an existing condition (of permanent differentiation between two races) is absolutely fundamental. Evidently, observation of the mere fact that permanent differentiations exist between races is a totally different matter from observation of the changes by which evolution occurs; it is compatible with almost any theory of the origin of diversities; for example, with that of special creation. As a matter of fact, do we find the existing races changing or permanent? What light does our study of variations throw on this?

In Paramecium, in the extensive study of many races for hundreds of generations by exact statistical and experimental methods, *not one single instance was observed of variation in the sense of an actual change in a race.* In the detailed paper, coefficients of variation are given almost by the hundred, and permanent diversities of race are registered minutely and in numbers. But these mean nothing so far as real change in any race is concerned. So far as the evidence goes, every race was essentially the same throughout the work, and may have been the same for unnumbered ages before.

For clear thinking it is of the greatest importance to distinguish variation as a process from variation as an existing static condition of diversity. If this distinction is not made, we may delude ourselves into thinking we have seen evolution occurring, when all we have seen is the complexity that induces us to invent the theory of evolution. The difference is just the difference between seeing a problem, and seeing its solution; between asking a question and answering it.

But is there indeed no evidence that actual racial changes occur in unicellular forms? On this point we have the extremely important work of Barber.[11] Barber was the first to undertake in bacteria and yeasts the study of "pure lines"—of races derived entirely from a single individual. In general his results were the same as those set forth above for Paramecium. Many races of yeasts and bacteria exist, and these races are constant (with the exceptions to be noted). Environmental effects were not inherited, and long continued selection was of no effect in changing such a race. Barber studied also unusual individuals; he found, just as I have set forth above for Parameicum, that their peculiarities were, as a rule, not inherited. But he did find a few cases of peculiar individuals *within a pure race*, that transmitted their peculiarities to their descendants. Here we have then actual changes in a race; variations in the dynamic sense. In this way there were produced races of yeasts having cells of a different form; races of bacteria composed of longer rods than the parents. But such cases were *extremely rare*. Variations that perpetuate themselves were found only in one individual among thousands. Barber's work goes as strongly as my own against the significance of the common variations among individuals—such variations as are measured by the coefficient of variation—for heredity or evolution.

To recapitulate, we find that the unicellular organisms are made up of numerous races, differing minutely but

[11] *Loc. cit.*

constantly. The individuals of any race vary much among themselves, but these differences are matters of growth and environment, and are not inherited. What is produced in reproduction depends on the fundamental constitution of the race, not on the peculiarities of the individual parent. The fundamental constitution of the race is resistant to all sorts of influences; it changes only in excessively rare instances, and for unknown causes; in a study of thousands of individuals of Paramecium, through hundreds of generations, hardly a single case of such change was observed.[12] Most differences between individuals are purely temporary and without significance in inheritance; the others are permanent diversities between constant races. Systematic and continued selection is without effect in a pure race, and in a mixture of races its effect consists in isolating the existing races, not in producing anything new.

To give in brief an account of the general results of extensive work, it is necessary to make definite statements, and to omit conditions, exceptions and qualifications. This the reader is asked to remember; the details may be found in the original papers. The results are based on study and measurements of more than 10,000 individuals of Paramecium, kept under experimental conditions for many generations. But science is essentially incomplete and its results at any time are not final. The author expects to make strenuous attempts to overthrow the generality of some of the results set forth.

[12] A single doubtful case is described in the first of the author's two papers: certain individuals of a race acquired a hereditary tendency to remain united after fission, while others did not show this tendency, or showed it less strongly.